Preliminary Communication

Soybean Curd Refuse Alleviates Experimental Tumorigenesis in Rat Colon

Naoyuki AZUMA, Hitoshi SUDA, Hiroyuki IWASAKI, Rhyuhei KANAMOTO, and Kimikazu IWAMI*

Department of Biological Resource Chemistry, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan

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Adult Fischer-344 rats which underwent administration of azoxymethane were fed diets containing soybean curd refuse (SCR) or a high-molecular-weight fraction of soy protein digest (HMF), or Hammarsten casein (CAS) as a protein source over a period of 34 weeks. All the living rats of each group at 22, 28 or 34 weeks were endoscopically inspected for tumor incidence in the colon. SCR turned out to be comparable to HMF in anti-tumorigenicity, or rather better than HMF.

Key words: soybean curd refuse; Fischer-344 rats; tumor incidence; per-anal endoscopy; anti-tumorigenicity

Soybean curd refuse (SCR), a byproduct in manufacturing tofu, a popular food in Japan, has been formerly eaten as a subsidiary article of diet. Recent alterations in life-style have made SCR unprofitable not only for human food but also for livestock feed. In these days, the "industrial waste" SCR is expensively disposed of by incineration at the risk of air pollution. SCR has abundant protein and dietary fiber, which occupied 20% and 10% in dry weight, respectively. SCR from which soy milk was pressed is still high in moisture content. This brings about hygienical or economic difficulties in extensive application of SCR as a practical foodstuff. At the first step toward the actualization of SCR use, SCR was examined for its preventive effect against tumorigenesis using a rat model of colon cancer.

This experimental design was approved by the Animal Experiment Committee of Kyoto Prefectural University and the laboratory animals were cared for in line with the ethical guideline for animal feeding. Male Fischer-344 rats aged beyond 6 months were divided into 3 groups (n=9), which underwent a total of 3 injections of azoxymethane in the abdominal cavity (15 mg/kg body weight once a week) for the first 3 weeks. Compositions of the respective test meals supplied for these 3 groups are given in Fig. 1, in which protein contents were adjusted to more than 8% in net protein ratio so as to fulfill the protein requirement for adult rats. The protein sources used here are as follows; SCR (dried okara "Propras-5005", a product of Fuji Oil Co., Osaka), an insoluble "high-molecular-weight" fraction centrifugally separable from microbial protease-treated soy protein isolate (HMF, Lot.950604 supplied by Fuji Oil Co.), and Hammarsten casein (CAS, purchased from Oriental Yeast Co., Tokyo). All the test meals contained 0.2% sodium deoxycholate as a cancer promoter, or else tumors did not occur in more than 30 weeks (our own observation). The Fischer-344 rats were individually housed in hanging stainless wire cages in an air-conditioned facility with a half-day light/dark cycle and were allowed free access to the respective diets and drinking water which were exchanged for fresh ones at intervals of a few days over a period of 34 weeks.

Figure 1 illustrates changes in body weight during the feeding period, and experimental schedules for per-anal endoscopy, blood drawing, and feces gathering. Since

Fig. 1. Experimentation Schedule, Change in Body Weight and Dietary Composition.

Fischer-344 rats weighing 300 g were treated with azoxymethane and fed three diets different in protein source. Arrows represent the weeks at which endoscopic observations on living rats were done.

* To whom correspondence should be addressed: Tel & Fax: +81-75-703-5661; E-mail, k_iwami@love.kpu.ac.jp

Abbreviations: SCR, soybean curd refuse; HMF, insoluble "high-molecular-weight" fraction after digestion of soy protein isolate with a microbial protease; CAS, Hammarsten casein
we had already observed that tumorigenesis was not detectable in the colon until the 20th week under quite similar conditions, endoscopic observation and blood or feces collecting were done at 22, 28, and 34 weeks. There were no significant differences in body weight gain among 3 groups through the feeding period of 34 weeks, during which a rat or more for each group died owing to such accidents as suffocation but never owing to carcinogenesis on dissection. For this reason, the object of tumor inspection was inevitably restricted to living rats at stated times. Before endoscopy, the rats were starved overnight. The feces for 3 days were collected before food deprivation and the blood was drawn from the tail vein between 9:00 and 10:00. Then, a Olympus BF-3C30 fiberscope (3.0 φmm in tip diameter) was carefully inserted into the colorectum under pentobarbital anesthesia.

The results of endoscopic observations at weeks 22, 28, and 34 are graphically depicted in Fig. 2, where tumor incidence was expressed in percentage as the ratio of tumor-bearing rats to total living ones for each group. At week 22, the number of living rats had been lessened by one in both CAS and SCR groups but tumorigenesis was perceived only in CAS group in which the tumor-bearing ratio of living rats stood at 2/8. At week 28, the number of living rats had come to 8 in common among 3 groups and tumorigenesis was aresh perceived in HMF group in which the tumor-bearing ratio of living rats stood at 1/8 versus 4/8 in CAS group. At week 34, tumorigenesis was perceived in all the groups in which the tumor-bearing ratio of living rats stood at 5/7 (CAS group), 1/7 (SCR group) or 2/8 (HMF group). Consequently, the tumor incidences at weeks 22, 28, and 34 were obtained in due order as 25.0, 50.0 and 71.4% (CAS group) or 0, 0 and 14.3% (SCR group) or 0, 12.5 and 25.0% (HMF group), respectively; the difference in tumor incidence between these CAS and SCR groups at week 28 was considered significant (P<0.05) by the Fisher’s exact probability test. Incidentally, the number of tumors per tumor-bearing rat was just 1.00 (n = 1 or 2) in all cases except 1.25 ± 0.24 (n = 4) at week 28 and 1.40 ± 0.24 (n = 5) at week 34 in the CAS group.

Dietary deoxycholate served as a risk factor promoting tumorigenesis in the colon. In this connection, bile acids were measured by the routine method using a commercially available assay kit (a product of Wako Pure Chemical Industries Ltd., Osaka) for their concentration in the plasma as well as their daily excretion into the feces at week 22, 28, or 34. The results of such measurements are summarized in the line graph in Fig. 3 (a,b). As for the plasma bile acid concentration, it was significantly higher in the CAS group than in the HMF or SCR group at week 22, 28, or 34 and a significant difference was also observed between the HMF and SCR groups in this respect. About one-fourth of HMF was looked upon as “resistant protein” not undergoing intestinal digestion and absorption, which is known to have an excellent capacity for binding bile acids and thereby to raise fecal bile acid excretion. Conversely, CAS relative to HMF may be said to facilitate intestinal bile acid reabsorption. The plasma bile acid concentrations in the CAS group was in inverse proportion to the amount of bile acids excreted into the feces. Meanwhile, the SCR group lay between the CAS and HMF groups in plasma bile acid concentration, despite its resemblance to the CAS group in fecal bile acid excretion. We have endoscopically perceived that HMF is more preventive against experimental tumorigenesis in rat colon than proteinous foodstuffs such as codfish meat, wheat gluten, and bovine milk casein (Azuma N. et al., in contribution elsewhere). Our observation here reveals that SCR
is equal or superior to HMF in anti-tumorigenicity. A characteristics of SCR different from HMF in proximate food composition is that SCR contains pectin-like hygroscopic fibers which swell fecal bulk in SCR-fed rats. Soft feces of large bulk imply embedment and dilution of carcinogens or smooth passage through the intestine even though accompanied with a somewhat unavoidable loss of nutrients. However, there are conflicting reports\textsuperscript{7-10} on whether or not dietary supplementation with pectin is valid for cancer prevention in rats. Accumulation of evidence is further required for a plausible explanation by which the anti-tumorigenic action of SCR somewhat surpassing HMF can be easily understood.

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References